DIFFUSION- AND REACTION RATE–LIMITED REDOX PROCESSES MEDIATED BY QUINONES THROUGH BILAYER LIPID MEMBRANES

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ABSTRACT The mediation of redox reactions through bilayer lipid membranes was studied. With an appropriate choice of electron acceptors the redox process can be limited either by the chemical reaction rate between the mediator and the reactants or by the shuttle frequency of the mediator through the membrane. Both modes were demonstrated for redox reactions mediated by 2,6 dichlorobenzoquinone (DCBQ) and by α-tocopherol with ascorbate entrapped inside vesicles using ferricyanide (a mild oxidant) or hexachloroiridate (a strong oxidant) in the external solution. The redox processes were reaction rate–limited and diffusion-limited for ferricyanide and hexachloroiridate, respectively. The kinetics of the redox processes in the diffusion- and the reaction rate–limited modes allows the determination of the shuttle frequencies and of the interfacial reaction rates of the mediators, respectively. The shuttle frequencies of DCBQ and α-tocopherol were ~8 and 0.08 s^{-1}, respectively, in L-α-dipalmitoyl phosphatidylcholine (DPPC) cholesterol vesicles at 25°C. Interfacial reaction rates between the mediators and ferricyanide were about two- and tenfold lower compared with bulk reaction rates for DCBQ (water) and tocopherol (50% ethanol solution), respectively, i.e., tocopherol is relatively less accessible to aqueous oxidants at the membrane interface. Tocopherol and oxidized tocopherol are reversible hydrophobic redox couples that interact very rapidly with strong oxidants. In both modes of mediation DCBQ was more effective than α-tocopherol.

INTRODUCTION

Redox reactions across bilayer lipid membranes mediated by various additives have been demonstrated by several investigators (see, for example, Hinkle, 1970; Hauska, 1977; Futami et al., 1979*a*, *b*; Runquist and Loach, 1981). A detailed analysis of a kinetic model has not been presented. However, in one of the above studies (Runquist and Loach, 1981), it was explicitly stated that the rate of the redox reaction was limited by the reaction at one side of the interface and not by the shuttle of the mediator across the membrane.

This paper deals theoretically and empirically with the mediation of redox reactions across lipid vesicles by quinone-like substances. The distinction between diffusion-limited and reaction rate–limited redox processes is a central theme of this presentation. Such an analysis enables the determination of the shuttle frequency of the mediator molecules across the membrane and of the rate constants of the reactions at the membrane interfaces. By comparing the latter with the rate constant of the reaction in bulk phases, it is possible to arrive at some conclusions regarding the protrusion of the reactive part of the mediator at the water–membrane interface.

THEORY

In the following model the oxidized and reduced forms of the mediator will be denoted by Q and QH_2, respectively. The total amount of the mediator, i.e., $Q + QH_2$, will be denoted by Q_t. The nominal concentration of the mediator added to the preparation will be denoted by brackets, e.g., $[Q_t]$, the concentration in the aqueous phase as $[Q]_w$ and $[QH_2]_w$, and its presence in the membrane will be expressed in terms of surface density (Q) and (QH_2). It is assumed that

$$(Q) = \beta[Q]_w$$

and

$$(QH_2) = \beta[QH_2]_w, \tag{1}$$

in which the constant β has the dimension of length transforming volume concentration to surface concentration.

The mediation of redox reactions through lipid membranes will be divided into apparent three stages: (*a*) The chemical reaction between the reduced mediator, QH_2, and the oxidant, Ox, at the oxidizing (outer) side of the vesicle. This stage can be further divided into both the reaction in the aqueous medium given by

$$aOx + QH_2(\text{aqueous}) \xrightarrow{k} Q(\text{aqueous}) + aRed, \tag{2a}$$

and the reaction with the mediator confined to the membrane interface,

$$aOx + Q\mathrm{H}_2(\text{membranous}) \xrightarrow{k'} Q(\text{membranous}) + aRed, \quad (2b)$$

in which a is a stoichiometric factor, usually 2, and *Red* is the reduced form of the oxidant. Although one molecule of reduced mediator eventually transfers two electrons, the rates of reactions 2a and 2b vary linearly with oxidant concentration rather than its square, since the intermediate semiquinone is highly reactive. The back-reactions of 2a and 2b are assumed to be negligible for the oxidants used in this study.

(*b*) The chemical reaction at the reducing (inner) side of the vesicle, which can be written in analogy to Eqs. 2a and 2b as follows:

$$Q(\text{aqueous}) + aRed' \xrightarrow{\bar{k}} Q\mathrm{H}_2(\text{aqueous}) + aOx' \quad (3a)$$

and

$$Q(\text{membranous}) + aRed' \xrightarrow{\bar{k}'} Q\mathrm{H}_2(\text{membranous}) + aOx' \quad (3b)$$

in which *Red'* and *Ox'* are the redox species present at the reducing side of the membrane. In this presentation the situation will be analyzed for the case in which the interior of the vesicles contains a strong reductant, *Red'*, at a relatively high concentration so that all the mediators in the inner side of the vesicles are in the reduced form, i.e., reactions 3a and 3b do not constitute a limit on the rate of the redox process.

(*c*) The shuttle of the mediator Q and $Q\mathrm{H}_2$, through the membrane, i.e.,

$$(Q)^{\text{out}} \overset{\ell}{\rightleftharpoons} (Q)^{\text{in}}$$

$$(Q\mathrm{H}_2)^{\text{out}} \overset{\ell}{\rightleftharpoons} (Q\mathrm{H}_2)^{\text{in}}, \quad (4)$$

where ℓ is the shuttle frequency. Superscripts "in" and "out" denote inner and outer sides of the vesicles, respectively.

In Eqs. 1 and 4, it is assumed that there is no difference between Q and $Q\mathrm{H}_2$ in terms of their interaction with the membrane, i.e., ℓ and β are assumed to be the same for Q and $Q\mathrm{H}_2$.

At a particular vesicle concentration the amount of the mediator adsorbed to the vesicle membrane area, A, constitutes a constant fraction, α, of the mediator added to the vesicle preparation. Therefore

$$[Q_t]_w = (1 - \alpha)[Q_t] \quad (5)$$

$$\text{Total } Q \text{ in membrane} = \alpha[Q_t]V \quad (6)$$

and

$$(Q_t) = \alpha[Q_t]V/2A, \quad (7)$$

in which $[Q_t]$ is the nominal mediator concentration in the vesicle preparation, V is the volume of the preparation, and (Q_t) is the surface concentration of $Q + Q\mathrm{H}_2$ at each interface. The factor 2 in Eq. 7 signifies that the total interfacial area is about twice the vesicle area. The factor α is a function of β and the total vesicle area. By dividing Eq. 5 by Eq. 7 and using Eq. 1 it follows that

$$\beta = \alpha V/2A(1 - \alpha), \quad (8)$$

from which an expression for α can be obtained, i.e.,

$$\alpha = 2A\beta/(V + 2A\beta). \quad (9)$$

The redox process will be analyzed next for the two extreme situations: a diffusion (or permeability)-limited process and a reaction rate–limited process.

Diffusion (or Permeability)-limited Process

This applies to the case in which

$$\alpha k'[Ox] + (1 - \alpha)k[Ox] \gg \ell. \quad (10)$$

This means that all the mediator in the outer side of the vesicle is in the oxidized form, Q. The rate of the redox process is determined, therefore, by the rate of arrival of the reduced mediator at the oxidizing interface, $\mathrm{d}Q\mathrm{H}_2/\mathrm{d}t$.

The rate that $Q\mathrm{H}_2$ molecules leave the reducing side of the vesicles is determined by the shuttle frequency, ℓ, and the surface concentration of $Q\mathrm{H}_2$ in the inner side of the membrane, $(Q\mathrm{H}_2)^{\text{in}}$, i.e.,

$$\mathrm{d}Q\mathrm{H}_2/\mathrm{d}t = \ell A(Q\mathrm{H}_2)^{\text{in}}. \quad (11)$$

Therefore, the rate of reduction of *Ox* is

$$-V\mathrm{d}[Ox]/\mathrm{d}t = a\ell A(Q\mathrm{H}_2)^{\text{in}}. \quad (12)$$

In a steady state the rate of $Q\mathrm{H}_2$ leaving the vesicles is equal to the rate of Q entering the vesicles. Therefore,

$$(Q\mathrm{H}_2)^{\text{in}} = (Q)^{\text{out}} = (Q_t). \quad (13)$$

Introducing Eq. 7 into Eq. 12, it follows that

$$-V\mathrm{d}[Ox]\mathrm{d}t = 0.5a[Q_t]\ell\alpha V. \quad (14)$$

Therefore,

$$\mathrm{d}[Ox]/\mathrm{d}t = -0.5\, a\ell\alpha[Q_t]. \quad (15)$$

Eq. 15 predicts a linear decrease of $[Ox]$ with time. As noted before the value of a is generally 2. If α can be determined independently the value of ℓ for a mediator can be calculated.

Except for a direct measurement of α, which can be performed in principle by straightforward dialytic experiments, Eq. 15 allows an estimate of α by comparing the

rate of the redox reaction in a diluted vesicle preparation with the rate at the original vesicle preparation. If $[Q_t]$ is kept constant the reaction rate should vary only through the change in α. From the dependence of α on the total vesicles area (see Eq. 9), it can be shown that

$$\alpha_f = \frac{1/f}{1/f + (1-\alpha)/\alpha}, \tag{16}$$

in which f is the factor of dilution and α_f is the value of α for the diluted preparation.

Reaction Rate–limited Process

This applies to the case in which

$$\ell \gg \alpha k'[Ox] + (1-\alpha)k[Ox]. \tag{17}$$

This condition implies that most of the mediator in the outer side of the membrane is in its reduced form. In a steady state the formation rate of Q in the bulk and at the interface is balanced by the entry rate of Q into the vesicles, which in turn is equal to the exit rate of QH_2 from the vesicles.

The reduction rate of the oxidant is determined by Eqs. 2a and 2b. If k' in Eq. 2b has the same dimensions as k, it follows that $k' \cdot [Ox] \cdot (QH_2)$ represents the change $d(QH_2)/dt$. To express the reaction 2b in terms of $d[Ox]/dt$, the above product should be multiplied not only by the factor a, but also by the ratio of total vesicles area to the volume of the vesicle preparation, i.e.,

$$d[Ox]/dt = -k'[Ox](QH_2) \cdot a \cdot (A/V). \tag{17a}$$

Since $(QH_2) = (Q_t)$, by introducing Eq. 7 into the above expression and adding the term due to reaction 2a, and taking into account Eq. 5, it follows that

$$-d[Ox]dt = ak[Ox][Q_t](1-\alpha) + 0.5\,ak'\alpha[Q_t][Ox]. \tag{18}$$

From Eq. 18 it follows that

$$-d\ln[Ox]/dt = [ak(1-\alpha) + 0.5\,ak'\alpha][Q_t]. \tag{19}$$

Integration of Eq. 19 leads to

$$[Ox] = [Ox]_0 \exp -(t/\tau), \tag{20}$$

in which

$$1/\tau = [ak(1-\alpha) + 0.5ak'\alpha][Q_t] \tag{21}$$

and $[Ox]_0$ is the concentration of the oxidant at time zero.

Since k can be determined by studying the reaction rate between the oxidant and the mediator in an aqueous solution, the value of k' can be calculated if α is known. For extremely hydrophobic mediators where $\alpha = 1$ Eq. 19 allows a direct estimate of k'.

Eq. 21 shows that $1/\tau$ varies linearly with the nominal mediator concentration $[Q_t]$. The change of $1/\tau$ with α depends on the difference between k and $0.5k'$. Thus, for $k > 0.5k'$, $1/\tau$ should increase with the decrease of α; this means that diluting the vesicles while keeping $[Q_t]$ constant should lead to a decrease in τ.

The model presented is not inconsistent with an equilibrium situation always prevailing between mediators in the bulk aqueous solution and those in the interface. Such an equilibrium is assumed to occur in the diffusion-limited processes. In the reaction rate–limited processes all that is required is that only a small fraction of the outside facing mediators are oxidized. This small fraction should give rise to an influx of oxidized mediators that is equal to the amount of oxidized mediators formed by the sum of reactions 2a and 2b. Although there is a realistic possibility that an equilibrium is established instantaneously between the interface and the bulk phase, there is nothing in the model or the results that excludes a nonequilibrium state.

METHODS

Liposomes made of DPPC (L-α-dipalmitoyl phosphatidylcholine) and cholesterol at a ratio of 10:1 were prepared using the injection method (Batzri and Korn, 1973); an ethanolic solution of the DPPC–cholesterol mixture was injected into a well stirred aqueous solution at a ratio of 1:12.5. Since DPPC has a transition temperature of 41°C the aqueous solution was preheated to a temperature over 60°C. The final DPPC concentration was 1.85 mM. The average liposome diameter was ~200 Å, as shown electron microscopically.

The outer solution was then changed using Sephadex G-50-80 columns following the method used by Radian and Kanner (1985). In this procedure 1-ml minicolumns were filled with the Sephadex preswollen by the solution planned to be outside. Before use, the minicolumns were placed in glass tubes and centrifuged at low speed (~1,500 *g*) for 1.5 min. Small portions of the liposome preparation (0.22 ml) were then carefully applied onto the precentrifuged columns which had been placed in clean glass tubes. Another centrifugation at the above speed and time yielded liposomes free of ethanol with the desired solutions inside and outside. In a sample of vesicle preparations the phospholipid concentration was determined before and after passage through the column and was found to be the same.

In the experiments in which ferricyanide was used as oxidant the inner solution contained 0.5 M ascorbic acid and 20 mM potassium phosphate adjusted with KOH to pH 5.8. The outer solution contained 0.5 M KCl and 20 mM potassium phosphate adjusted to the same pH. When hexachloroiridate was used as an oxidant the inner solution contained 0.5 M ascorbic acid and 20 mM potassium acetate adjusted with KOH to pH 5.0. The outer solution contained 0.5 KCl and 20 mM potassium acetate adjusted to the same pH.

α-Tocopherol was dissolved in the ethanolic liposome-forming solution. Its final concentration in the liposome preparation varied between 5×10^{-5} and 2×10^{-4} M as indicated in the figures and tables. The spectrum of the α-tocopherol–containing vesicles showed a clear peak at 292 nm. From the size of this peak it was inferred that tocopherol concentration after passage through the column was at least 90% of the original concentration. The reductive capacity of the vesicle preparations with and without tocopherol were about the same. It is inferred, therefore, that tocopherol did not affect significantly the size of the vesicles.

A 2,6 dichlorobenzoquinone (DCBQ) aqueous solution was prepared as follows: a portion of DCBQ ethanolic solution was dried in a glass tube with a stream of N_2. The desired outer solution was then added and was well stirred. Small amounts of this solution were applied to the already formed liposome preparation. DCBQ concentration varied between 5×10^{-7} and 5×10^{-5} M as indicated in the figure legends and tables.

DPPC, cholesterol, and α-tocopherol were purchased from Sigma

Chemical Co., (St. Louis, MO), potassium hexachloroiridate from Alfa Scientific Inc. (Hayward, CA), and DCBQ from Eastman Kodak Co. (Rochester, NY). The reduction of ferricyanide and hexachloroiridate were followed by a spectrophotometer (Uvikon 810, Kontron Analytical, Everett, MA) at 420 nm and 487 nm, respectively. The reactions were carried out at temperatures of 25°–27°C.

RESULTS

I. Hexachloroiridate–Ascorbate Redox Reactions Mediated by DCBQ and α-Tocopherol

To satisfy the conditions for a diffusion-limited rate process the strong oxidizer hexachloroiridate, $Ir(Cl)_6^{--}$, was used as an oxidant. Fig. 1 depicts the reduction of hexachloroiridate with time. It can be seen that with the addition of DCBQ the reduction proceeds linearly with time and that linearity is maintained for consecutive additions of the oxidant. It is clear from Fig. 1 that hexachloroiridate disappears exponentially even in the absence of any defined mediator. Although this reaction is significant compared with the stability of hexachloroiridate in an aqueous solution of the same composition, it is small compared with the mediated redox reaction.

Fig. 2 shows the reduction of hexachloroiridate mediated by α-tocopherol. Here too, reduction proceeds linearly with time, and linearity is maintained until the internal ascorbate is exhausted. Table I summarizes the results for the rates of redox reactions between hexachloroiridate and ascorbate mediated by DCBQ or tocopherol for various mediator and lipid concentrations. Note that in diluting the vesicles by the factors of 5 and 20 and keeping

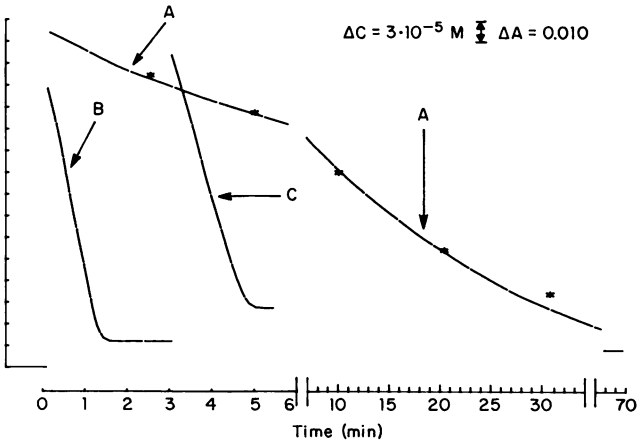

FIGURE 1 Concentration change in hexachloroiridate as monitored by the decrease in absorbance at 487 nm after the addition of potassium hexachloroiridate at ~0.5 mM to a vesicle preparation containing 0.5 M ascorbate inside the vesicles. Curve *A* represents the results without any mediator. Curves *B* and *C* represent the absorbance change after consecutive additions of hexachloroiridate to the same preparation in the presence of 5×10^{-7} M DCBQ. The points on curve *A* correspond to an exponential decrease in concentration with a time constant of 20 min. At the end of the reaction there is a small constant increment in absorbance relative to the initial value. The vesicle preparations contained DPPC at 1.85 mM and cholesterol at 0.18 mM.

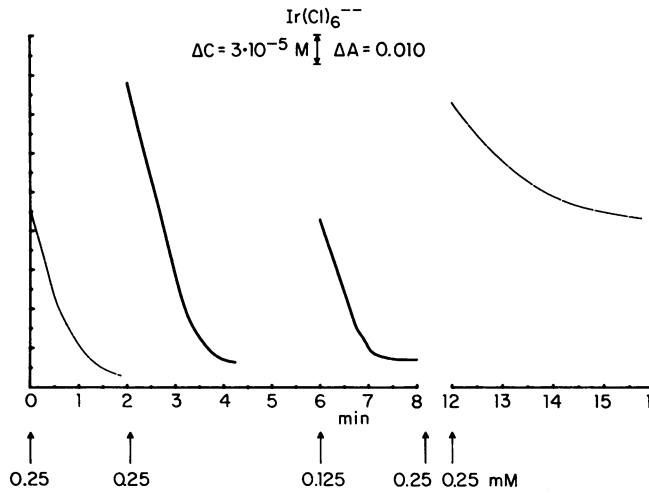

FIGURE 2 Time course of reduction of hexachloroiridate mediated by α-tocopherol after consecutive additions (arrows) of the oxidant to a vesicle preparation containing 0.5 M ascorbate inside the vesicles. The amount of hexachloroiridate added is indicated in terms of its concentration in the preparation. The vesicle preparation contained DPPC at 1.85 mM, cholesterol at 0.18 mM, and α-tocopherol at 0.2 mM.

the DCBQ concentration constant, the rate of the redox process decreased only by the factors of 0.62 and 0.22, respectively. According to Eq. 15 this means that a dilution by the factors of 5 and 20 led to a decrease of α by 0.62 and 0.22, respectively. By Eq. 16 it can be shown that this corresponds to a value for α of 0.83 at 1.85 mM DPPC.

When the values of α and $[Q_t]$ are introduced into Eq. 15, the value of ℓ for DCBQ becomes ~8/s. The value of α for tocopherol is very close to 1. The shuttle frequency, ℓ, for tocopherol according to the figures presented in Table I is, therefore, ~0.08/s.

II. Ferricyanide–Ascorbate Redox Reactions Mediated by DCBQ and α-Tocopherol

Fig. 3 shows typical reduction experiments of ferricyanide in the presence of DCBQ. Curve *A* shows that in the

TABLE I
REDUCTION RATES OF HEXACHLOROIRIDATE IN VESICLE PREPARATIONS CONTAINING 0.5 M POTASSIUM ASCORBATE

Mediator	Concentration	[DPPC]	α	d[*Ox*]/d*t*
	M	*mM*		10^{-9} *mol/cm³ · s*
DCBQ	5×10^{-7}	1.85	0.83*	3.5 ± 0.66 (24)
DCBQ	5×10^{-7}	0.37	0.49*	2.17 ± 0.28 (3)
DCBQ	5×10^{-7}	0.1	0.196*	0.76 ± 0.08 (3)
α-Tocopherol	5×10^{-5}	1.85		3.9 ± 0.5 (5)
α-Tocopherol	10^{-5}	1.85		0.95 (2)

The outer medium contained 0.5 M KCl. Both sides contained 20 mM acetate at pH 5.0. Numbers in parentheses denote number of determinations. ±, standard deviations.
*Calculated values according to Eqs. 15 and 16.

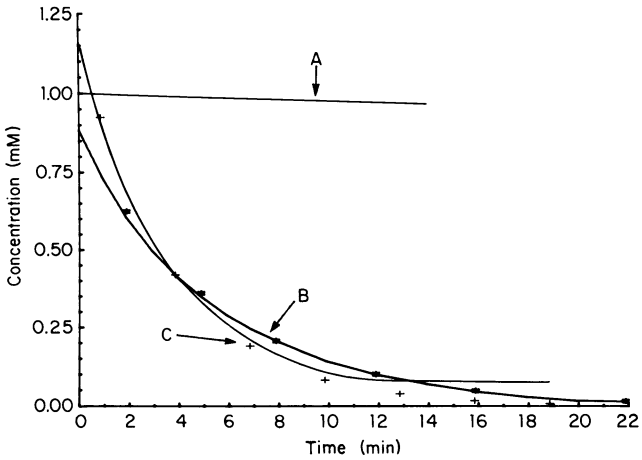

FIGURE 3 Time course of reduction of ferricyanide mediated by DCBQ as monitored by the change in absorbance at 420 nm after its addition to a vesicle preparation containing 0.5 M ascorbate inside the vesicles. Note that in the absence of the mediator the reaction is negligible. Curves *B* and *C* represent results of experiments in which the amount of ferricyanide added was less or more, respectively, as compared with the equivalent amount of ascorbate inside the vesicles. The points on the curves represent a calculated exponential course with time constants of 4.8 and 4.0 min for curves *B* and *C*, respectively. Note that curve *B* is a single exponential throughout the entire range.

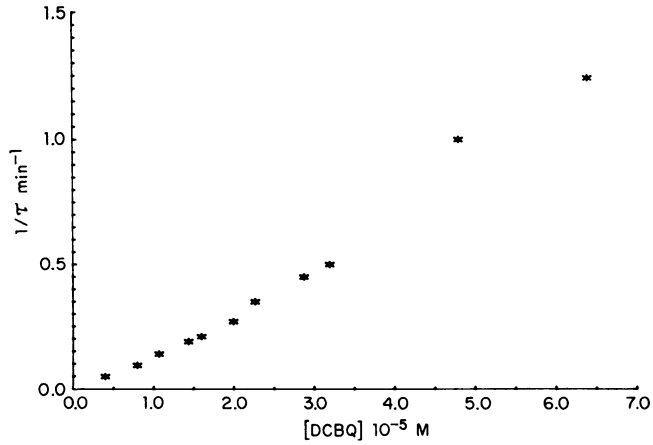

FIGURE 4 The reciprocal of the time constant of ferricyanide reduction by ascorbate-containing vesicles as a function of DCBQ concentration. The results were obtained for a particular vesicle preparation containing 1.85 mM DPPC and 0.18 mM cholesterol.

absence of the quinone the decline of ferricyanide is extremely slow. Curves *B* and *C* represent experiments in which the amount of ferricyanide added to the liposome solution was less or more, respectively, compared with the equivalent amount of ascorbate present inside the vesicles. It can be seen that for the case where ascorbate is in excess the decline of ferricyanide in the solution follows an exponential course throughout the entire reaction. On the other hand when ferricyanide is in excess the reaction rate toward the end of the process decreases relative to the initial rate. This is of course expected since under these conditions, as the internal ascorbate is exhausted, the reaction at the reducing interface becomes rate limiting. These results validate the assumption that as long as the ascorbate concentration does not drop below ~50 mM, the reduction of the quinone at the reducing interface is not rate limiting. The "pure" exponential nature of curve *B* implies also that the vesicles in the preparation were quite homogeneous in terms of an area/volume ratio, a fact that corresponds to the electron microscopic picture of the vesicles.

Fig. 4 shows the results of several experiments in which the time constant of the exponential decay of ferricyanide was measured. As expected from Eq. 21 the time constant, τ, of the redox reaction process is inversely proportional to the nominal concentration of DCBQ in the solution.

An example for a tocopherol-mediated redox process with ferricyanide is shown in Fig. 5.

Table II summarizes the results of the time constant determinations of redox reactions between ascorbate and ferricyanide mediated by DCBQ and tocopherol.

It is noteworthy that the time constant of DCBQ-mediated reactions decreases when the vesicles are diluted. This should happen according to Eq. 21 if $k > 0.5'$. The rate constant of the reaction between ferricyanide and DCBQ in water, k, was found to be ~400 $M^{-1} s^{-1}$. Using the value of τ from Table II and the value of α (0.83) from Table I, the value of k' according to Eq. 21 is ~100 $M^{-1} s^{-1}$.

A factor of 2 between k and k' is to be expected since a mediator in the aqueous solution can be approached by a ferricyanide molecule from a spatial angle of 4π steradians compared with a spatial angle of 2π steradians for an interfacially confined mediator. Thus it can be concluded that the interfacial reaction rate between DCBQ and ferricyanide is only slightly less than the reaction rate in the aqueous solution.

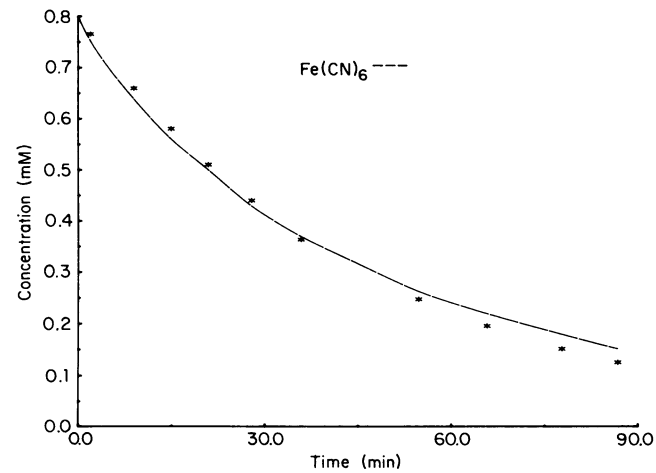

FIGURE 5 Time course of α-tocopherol–mediated ferricyanide reduction by ascorbate-containing vesicles. The mediator was included in the vesicle membranes at a final concentration of 0.2 mM. The other constituents of the vesicle membrane were DPPC at 1.85 mM and cholesterol at 0.18 mM.

TABLE II
TIME CONSTANT, τ, FOR THE EXPONENTIAL DECREASE OF FERRICYANIDE IN VESICLE SOLUTIONS CONTAINING 0.5 M POTASSIUM ASCORBATE INSIDE THE VESICLES

Mediator	Concentration	DPPC concentration	τ
	M	*mM*	*min*
DCBQ	2×10^{-5}	1.85	3.7 ± 0.3 (7)
DCBQ	2×10^{-5}	0.37	2.76 ± 0.5 (5)
α-Tocopherol	2×10^{-4}	1.85	44.5 ± 3.0 (3)
α-Tocopherol	5×10^{-5}	0.37	86.0 ± 6.0 (3)

The outer medium contained 0.5 M KCl. Both solutions contained 20 mM phosphate at pH 5.7. Numbers in parentheses denote number of determinations. ±, standard deviations.

The reaction rate constant between tocopherol and ferricyanide, k', is, according to the results presented in Table II, only ~2 M^{-1} s^{-1}, i.e., almost two orders of magnitude smaller than the value of DCBQ.

We have studied the reaction rate constant between reduced tocopherol and ferricyanide in 50% ethanol solution. The rate constant was found to be 40 M^{-1} s^{-1}. Thus the ratio between the interfacial reaction rate for tocopherol vs. that for DCBQ reveals a fivefold discrepancy, indicating that the interfacial reactive part of tocopherol is less accessible to reaction with ferricyanide in comparison to DCBQ.

DISCUSSION

The results presented in this study demonstrate a clear distinction between reaction rate–limited and diffusion-limited mediation of redox processes through lipid membranes. This distinction is shown for two types of mediators, a small quinone and α-tocopherol. Comparing the mediation by these compounds we did not find an unusual phenomenon like that described by Hauska and his colleagues (Hauska, 1977; Futami et al., 1979*a*). They showed that tocopherol- and isoprenoid-containing quinones were much more effective in mediating redox processes than were small molecular weight quinones that do not contain isoprenoid tails. The opposite is found in our study. Thus in the diffusion-limited mode of reaction (ascorbate, in; hexachloroiridate, out) the calculated ℓ is ~100-fold higher for DCBQ than for α-tocopherol. In the reaction rate–limited mode (ascorbate vs. ferricyanide) the calculated interfacial reaction rate between ferricyanide and the reduced DCBQ is higher by a factor of ~50, being ~100 and 2 M^{-1} s^{-1} for DCBQ and tocopherol, respectively. Therefore, in both modes, DCBQ was much more potent as a mediator than was tocopherol. A rigorous comparison between the results of the experiments reported in this study and those of Futami et al. (1979*a*) is not possible because of the difference in the nature of lipids used and the preparation method of vesicles. Yet, by comparing the data that appeared in Table I of Futami et al. (1979*a*) at a mediator concentration of ~5×10^{-4} M and converting it according to Eq. 15 to levels of concentration used in our study for the diffusion limited–mode of mediation, it can be gathered that the difference between these two studies relates primarily to the small molecular weight quinones; their efficacy as mediators in the system of Futami et al. is at least three orders of magnitude lower than that of DCBQ in our system. It should be noted that in the study by Futami et al. the reductant was dithionite rather than ascorbate. It is possible that the interaction between the strong reductant dithionite and quinone mediators in the aqueous solution produces hyperreduced species that are either "destroyed" irreversibly in the water or extracted from the membrane because of their hydrophilic nature.

A significant result of this study is related to the clear redox mediation effect of tocopherol. It should be stressed that ubiquinones (we tried ubiquinone-6 and ubiquinone-10) were hardly effective in mediating redox processes in our system, most probably due to their low redox potential which precluded a reaction (or a fast enough reaction) with ascorbate. On the other hand tocopherol was readily reduced by ascorbate. It is implied, therefore, that tocopherol forms an effective hydrophobic reversible redox species with a redox potential higher than zero and lower than 0.4 V (vs. standard hydrogen electrode). Thermodynamically it can be reduced by cellular reductants such as glutathione. The fact that its interfacial rate of reaction with ferricyanide is significantly lower than the bulk rate-constant, implies that it is available in its reduced form in the interior of the membrane. Therefore reduced tocopherol can act as a membranal antioxidant agent. The implication from this study is that the antioxidant potency of tocopherol can be restored by occasional interfacial reactions with appropriate aqueous reductants. To assess the significance of interfacial redox reactions of tocopherol in biological membranes it is necessary to study its interaction with glutathione, reduced NAD and NADP, and ascorbate at physiological concentrations. These topics are being explored in our laboratory.

The study of redox reaction mediation in lipid vesicles constitutes a new way of probing molecular dynamics in the bilayer structure. Thus, the study of diffusion-limited processes can yield the value of transmembrane shuttle frequencies. It is noteworthy that for DCBQ the figure is very small; only ~8 s^{-1}. For a membrane ~30 Å thick, this corresponds to a nominal diffusion coefficient lower than 10^{-12} cm^2/s. The lateral diffusion coefficient of pyrene in the same type of membrane at the same temperature was found to be 10^{-9} cm^2/s (Daems et al., 1985). Thus we are led to the conclusion that DCBQ is concentrated mostly in the interfacial region of the bilayer and is excluded from the lipid bilayer interior by a significant barrier. This is consistent with the fact that k' for DCBQ is only slightly

less than that expected from the bulk water reaction rate, k (see Results). This indicates that the quinones in the membrane are readily available for direct oxidative attack by an aqueous oxidant. If they were mostly dissolved in the bulk lipid phase the value of k' should have been much smaller than k. This is also in accord with the observation of the high α value for DCBQ (see Table I), which nominally corresponds to a bulk partition coefficient between hydrophobic solvent and water of more than 1,000, whereas the actual number for benzene-type molecules is only in the range of 10–30 (Katz and Diamond, 1974). The notion that quinones are mostly adsorbed at the membrane–water interface was already suggested by a completely independent observation regarding photodriven electron transport from porphyrin to quinone in lipid bilayer membranes (Krakover et al., 1981).

Another feature that can be explored by these studies is the relative availability of the active moiety of the mediator in the membrane to an aqueous reactant. This can be achieved by a careful comparison between the bimolecular reaction rate at the membrane interface with the reaction rate between the two species in bulk phases. As noted in this study it is inferred that the active reduced form of α-tocopherol is relatively less accessible to oxidation by an aqueous oxidant than is DCBQ.

This work was supported by a grant from Dr. Fred S. Singer through The Authority for Research and Development of the Hebrew University. T. Krakover is a recepient of the postdoctoral award from the Jacob and Mini Bacaner foundation.

Received for publication 15 April 1986 and in final form 31 July 1986.

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